

## VII. CLAIMING

What is claimed is:

1. The procedure for cloning human  $\beta$ A precursor protein gene (human APP gene)  
based on the reverse transcription (RT) and the polymerase chain reaction (PCR)  
5 using the synthesized oligonucleotides (SEQ ID NO. 1) for RT, and (SEQ ID NO. 2)  
and (SEQ ID NO. 3) respectively for PCR, comprising:
  - Isolating RNA.
  - Performing RT reaction using the synthesized oligonucleotide  
5' GTTACAGCACAG 3' (SEQ ID NO. 1) under the following  
10 conditions: 90<sup>0</sup>C for 2 minutes; 0<sup>0</sup>C for 1 minute; 25<sup>0</sup>C for 10  
minutes; 42<sup>0</sup>C for 45 minutes;
  - Performing PCR reaction using the synthesized oligonucleotides  
5' ATGCTGCCCCGGTTTGGC 3' (SEQ ID NO. 2) and  
5' CTAGTTCTGCATCTGCTCA 3' (SEQ ID NO. 3) under the  
15 following conditions: Denaturing at 94<sup>0</sup>C for 1 minutes; annealing at  
55<sup>0</sup>C for 2 minutes; elongating at 72<sup>0</sup>C for 3 minutes each cycle, for 35  
cycles.
2. The procedure for the construction of expression plasmids using the pFastBac<sup>TM</sup> HTb  
20 and the pBlueBacHis2 A transfer vectors for the purpose of obtaining human APP in  
insect cells, comprising:
  - 2.1. Using the pFastBac<sup>TM</sup> HTb vector:
    - Digesting the pFastBac<sup>TM</sup> HTb vector with XbaI and HindIII followed by  
dephosphorylation with calf intestinal alkaline phosphatase;

- Digesting the vectors (1) pCR<sup>R</sup> II/APP<sub>751</sub>-cDNA and (2) pCR<sup>R</sup> II/APP<sub>770</sub>-cDNA with XbaI and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP<sub>751</sub>-cDNA and APP<sub>770</sub>-cDNA;
- Ligating the APP<sub>751</sub>-cDNA and APP<sub>770</sub>-cDNA fragments to the pFastBac<sup>TM</sup> HTb vectors and introducing the ligation products in INVαF' E. Coli strain;
- Screening for inserts based on the presence of white colonies, as a result of which the vectors (3) pFastBac<sup>TM</sup> HTb /APP<sub>751</sub>-cDNA and (4) pFastBac<sup>TM</sup> HTb /APP<sub>770</sub>-cDNA are selected;
- Introducing the vectors (3) and (4) in DH10Bac<sup>TM</sup> E. Coli competent cells;
- Screening for recombinant bacmids in DH10Bac<sup>TM</sup> E. Coli using blue-white color selection, then verifying the presence of APP-cDNA's inserts in the recombinant bacmids by PCR amplification using the M13 forward (-40) and M13 reverse primers, as a result of which the recombinant bacmids (5) for vectors (3) in DH10Bac<sup>TM</sup> E. Coli and (6) for vector (4) in DH10Bac<sup>TM</sup> E. Coli respectively are selected;

## 2.2. Using the pBlueBacHis2 A vector:

- Digesting the pBlueBacHis2 A vector with NcoI and HindIII followed by dephosphorylation with calf intestinal phosphatase;
- Digesting the vectors (3) pFastBac<sup>TM</sup> HTb/APP<sub>751</sub>-cDNA and (4) pFastBac<sup>TM</sup> HTb/APP<sub>770</sub>-cDNA with NcoI and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP<sub>751</sub>-cDNA and APP<sub>770</sub>-cDNA;
- Ligating the APP<sub>751</sub>-cDNA and APP<sub>770</sub>-cDNA fragments to the pBlueBacHis2 A vectors and introducing the ligation products in INVαF' E. Coli strain;

- Screening for inserts using blue-white color selection, as a result of which the vectors (7) pBlueBacHis2 A/APP<sub>751</sub>-cDNA and (8) pBlueBacHis2 A/APP<sub>770</sub>-cDNA are selected.

5     3.     The procedure for the construction of expression plasmids using the pET-28a (+) transfer vector for the purpose of obtaining human APP in bacteria, comprising:

- Digesting the pET-28a (+) vector with Sall and HindIII followed by dephosphorylation with calf intestinal alkaline phosphatase;

10     - Digesting the vector (3) pFastBac<sup>TM</sup> HTb/APP<sub>751</sub>-cDNA and (4) pFastBac<sup>TM</sup> HTb/APP<sub>770</sub>-cDNA with Sall and HindIII and isolating the resulting fragments containing the cDNA coding sequences of APP, APP<sub>751</sub>-cDNA and APP<sub>770</sub>-cDNA;

- Ligating the APP<sub>751</sub>-cDNA and APP<sub>770</sub>-cDNA fragments to the pET-28a (+) vectors and introducing the ligation products in INVαF' E. Coli strain;

15     - Screening for inserts based on the presence of white colonies, as a result of which the vectors (9) pET-28a (+)/APP<sub>751</sub>-cDNA and (10) pET-28a (+)/APP<sub>770</sub>-cDNA are selected.

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## VIII. REFERENCES

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